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22

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T cell receptor gene-therapy has entered the clinic and shown potential for successful cancer treatment. However, the clinical evaluation has also highlighted the need for selection of truly cancer-specific targets. Merkel cell carcinoma (MCC) is a highly aggressive skin cancer associated with Merkel cell polyomavirus (MCPyV). Due to the clear viral correlation CD8+ T cells specific for viral epitopes could potentially form cancer-specific targets in MCC patients. We have identified MCPyV specific T cells using a high-throughput platform for T-cell enrichment and combinatorial encoding of fluorescence-labeled major histocompatibility complex (MHC) class I multimers. We identified 35 T cell epitopes among 398 MCPyV derived peptides analyzed. Strikingly, T-cell responses against the two oncogenic MCPyV proteins Large T antigen and small T antigen were exclusively present in blood of MCC patients when compared to healthy donors. We demonstrate both the processing and presentation of oncoprotein-derived epitopes, as well as lytic activity of specific T cells towards MHC-matched MCC cells. Demonstrating the presence of oncoprotein-specific T cells among tumor infiltrating lymphocytes *ex vivo* further substantiated the relevance of the identified epitopes. The viral epitopes represents specific targets and should be ideal for TCR-gene therapy approaches. We have isolated and sequenced MCPyV oncogenic protein specific
T cell receptors and are currently testing *in vitro* transduction systems with the purpose of introducing the TCRs into human PBMC, injecting them into immune deficient NOG mice carrying HLA matched MCPyV positive tumor to investigate the tumor rejection capacity of these gene-modified T cells.

23

Selection process of the optimal T-iPSC clone from among clones derived from T cells specific to melanoma antigen MART-1

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We have previously reported that iPS cells (iPSCs) were established from mature cytotoxic T cells specific to MART-1 antigen from a melanoma patient, and that CD8+ mature T cells were generated from these iPSCs (Cell Stem Cell, 2013). This method represents a novel tool for the cloning and expansion of T cells that can be applied for cell therapy against cancer. Whereas this approach has been based on the idea of autologous transplantation, we are also thinking of applying this method to the allogeneic transplantation settings.
In this study, we tried to established a method to select optimal T-iPSC clone from multiple clones. We firstly expanded MART-1 specific CD8+ T cells from a healthy donor, and then reprogrammed these cells using Yamanaka factors. A total of 8 iPSC clones were established, and regenerated T cells from these clones were found to express different T cell receptor (TCR). We found that the affinity of these TCR varied very widely, and accordingly the difference was observed in the cytotoxic activity of regenerated T cells. We also found that these iPSC clones were intrinsically very heterogeneous in terms of the efficiency in the in vitro regeneration of T cells. These findings propose that selection process of the best clone among multiple T-iPSC clones is required for this strategy. We are now testing allo-reactivity of regenerated T cells, which would be a risk in allogeneic transplantation setting. Such information in total will be very important for the development of fundamental technology in this strategy.

